



RESEARCH

Differing proteome responses to ocean acidification between two common pocilloporid corals

Marleen Stühr¹ · Laxmikanth Kollipara² · Claire E. Reymond^{1,3,4,5} · Dirk de Beer⁶ · Justin Ries^{1,7} · Albert Sickmann^{2,8} · Hildegard Westphal^{1,9}

Received: 19 June 2025 / Accepted: 23 November 2025
© The Author(s) 2025

Abstract Ocean acidification threatens coral reef ecosystems by challenging calcification processes fundamental to reef accretion. Yet many corals continue to calcify under elevated $p\text{CO}_2$, suggesting species-specific physiological plasticity and potential cellular compensations. Here, we use label-free quantitative proteomics to investigate proteomic responses of two common pocilloporid corals, *Stylophora pistillata* and *Pocillopora damicornis*, with known differential resistance to ocean acidification after two months at moderate (~940 ppm) and high (~2,800 ppm) $p\text{CO}_2$ compared to the control (~480 ppm). *S. pistillata* exhibited extensive proteomic restructuring under high $p\text{CO}_2$, marked by widespread declines of energy-generating pathways, yet selective increase of proteins involved in ion transport, cytoskeletal stability, and stress responses. This indicates a strategy of general metabolic suppression coupled with targeted investment into essential cellular functions, potentially sustaining calcification despite reduced

overall metabolic capacity. In contrast, *P. damicornis* showed much less proteomic adjustment, primarily involving structural proteins and those potentially linked to cellular redox balance, signifying a moderate, targeted strategy for physiological stability. These divergent responses highlight contrasting modes of resistance (plasticity versus stability). Integrated with physiological data, our findings clarify cellular mechanisms controlling calcification, demonstrating the value of proteomics in coral ecophysiology and providing new insights into species-specific vulnerability under future ocean conditions.

Keywords Climate change · CO_2 · LC–MS/MS proteomics · Coral calcification · *Stylophora pistillata* · *Pocillopora damicornis*

Introduction

Coral reefs face severe threat from global climatic and oceanic change, particularly from warming and ocean acidification (OA)(IPCC 2022). Rising partial pressure

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00338-025-02801-y>.

✉ Marleen Stühr
marleen.stuhr@leibniz-zmt.de

¹ Leibniz Centre for Tropical Marine Research (ZMT),
28359 Bremen, Germany

² Leibniz-Institut für Analytische Wissenschaften – ISAS –
e.V., 44139 Dortmund, Germany

³ School of Geosciences, University of Sydney, Sydney,
NSW 2006, Australia

⁴ School of Life and Environmental Sciences, University
of Sydney, Sydney, NSW 2006, Australia

⁵ Marine Studies Institute (MSI), University of Sydney,
Sydney, NSW 2006, Australia

⁶ Department of Molecular Ecology, Max-Planck-Institute
for Marine Microbiology, Bremen, Germany

⁷ Department of Marine & Environmental Sciences, Marine
Science Center, Northeastern University, Nahant, MA 01908,
USA

⁸ Medizinische Fakultät, Ruhr-Universität Bochum, Bochum,
Germany

⁹ Department for Geosciences, Bremen University, Bremen,
Germany

of atmospheric carbon dioxide ($p\text{CO}_2$) concentrations drive increases in seawater $p\text{CO}_2$, lowering pH and altering carbonate chemistry at rates that may exceed many reef-building species' ability to acclimatize (Liew et al. 2018; Comeau et al. 2019). While warming effects are evident in tropical coral reefs, OA effects will intensify as atmospheric $p\text{CO}_2$ exceeds 1,000 ppm by century's end of this century (IPCC 2022), leading to ocean pH decline by 0.3 to 0.4 units, altering seawater chemistry and impairing the ability of marine calcifiers to form skeletons and shells (Caldeira and Wickett 2003; Orr et al. 2005; Kawahata et al. 2019). As ocean chemistry shifts under elevated $p\text{CO}_2$, shallow tropical marine habitats face increasing stress, coral reefs' geo-ecological role through carbonate framework and sediment deposition (Hoegh-Guldberg et al. 2017; Cornwall et al. 2021), vital for maintaining reef structure, biodiversity, and supporting coastal communities worldwide (Costanza et al. 1997).

Calcifying marine organisms such as corals, foraminifera, echinoderms, and mollusks (Ries et al. 2009; Kawahata et al. 2019; Khalil et al. 2022; Narayan et al. 2022) are considered particularly vulnerable to OA, as it impedes calcification and alters skeletal microstructures (Stuhr et al. 2021; Khalil et al. 2022). These effects arise from a dual mechanism: increased energetic costs to remove excess protons (H^+) from the calcifying fluid for skeleton formation (Cohen and Holcomb 2009; Guillermic et al. 2021), and disruption of pH homeostasis at the tissue-skeleton interface, which can compromise calcification and activity of key enzymes (Mollica et al. 2018). Understanding these effects on reef-building organisms, such as coralline algae and scleractinian corals (Comeau et al. 2019; Kawahata et al. 2019; Cornwall et al. 2021), is essential, as they provide the scaffolding for coral reefs and the essential ecosystem services they provide.

Studies on OA effects on tropical corals found diverse responses between host and symbiont species (Ries et al. 2009; Hoadley et al. 2015; Hoegh-Guldberg et al. 2017; Kawahata et al. 2019; Cornwall et al. 2021), due to complex biological impacts on photo-physiology, calcification and pH regulation (Cornwall et al. 2024). The effects on photosymbiont-bearing calcifiers often appear limited or positive, suggesting remarkable resilience (Ries et al. 2009; Leung et al. 2022). This is supported by CO_2 's 'fertilizing' effect, enhancing availability of CO_2 to photosynthesizing microalgae within the coral tissue, boosting their productivity and photosynthate supply (Tremblay et al. 2013) to cnidarian hosts. This may provide energy to compensate for increased gradients between calcifying fluid and surrounding seawater ($\Delta[\text{H}^+]$) (Ries et al. 2009; Gold and Vermeij 2023). Moreover, proteins that facilitate organic matrix organization appear crucial for calcification by lowering costs of growth energy (Tambutté et al. 2015; Zaquin et al. 2022). Nevertheless, our understanding of molecular processes

behind corals' capacity to adjust to acidified conditions and continued calcification remains incomplete.

A two-month experiment showed enhanced calcification rates of three tropical corals with increasing $p\text{CO}_2$ up to 3,000 ppm at control temperatures (Fig. 1; Guillermic et al. 2021; Cameron et al. 2022). However, their ability to elevate the pH of the calcifying fluid (pH_{CF}) was impaired under thermal stress, reducing calcification rates. This pH_{CF} decline correlated with symbiont loss, linking photosynthetic energy availability and pH regulation; however, molecular mechanisms for elevated proton removal remain unclear. High-throughput omics approaches can help clarify these pathways (Cziesielski et al. 2018; Stuhr et al. 2018; Williams et al. 2023) and has proven valuable in studying calcification and host-photosymbiont interactions in large benthic foraminifera (Stuhr et al. 2021) where high $p\text{CO}_2$ triggered significant changes in stress, metabolic, and cellular maintenance proteins. These highlight cellular challenges faced by calcifying organisms, where energy diversion toward calcification or stress management may affect other physiological functions.

Proteomics is an essential tool for understanding physiological processes, as proteins directly drive cellular functions and represent phenotypic responses at the cellular level (Diz et al. 2012). Despite its potential, few studies have employed high-throughput proteomics to investigate protein dynamics in corals (Maor-Landaw and Levy 2016; Williams et al. 2023), especially under OA (Lin et al. 2022). Coral proteomics studies have revealed that protein-level responses often differ from transcriptomic patterns (Mayfield et al. 2016; Cziesielski et al. 2018), highlighting the importance of regulatory mechanisms (post-transcriptional modifications, protein degradation) in response to environmental changes (Diz et al. 2012). Proteomics data provide insights into cellular response dynamics and reveals adaptive mechanisms undetected by conventional methods. Moreover, recent studies have shown that coral protein signatures can predict their susceptibility (Mayfield et al. 2022) and that proteome-based clustering can resolve relationships among reef holobionts, providing phylogenetic context and uncovering cryptic diversity (Stuhr et al. 2022).

By investigating and comparing the proteomic responses of two ecologically significant tropical coral species, *Stylophora pistillata* and *Pocillopora damicornis*, to simulated OA scenarios, this study aims to identify key proteins involved in coral acclimatization and stress response to OA—addressing how corals continue calcifying under severe OA. Both pocilloporids belong to the scleractinian 'robust' clade. While *S. pistillata* is considered resistant to OA, *P. damicornis* has shown inconsistent responses to elevated $p\text{CO}_2$, with lower capacity to regulate the extracellular calcifying medium (Venn et al. 2019 and references therein), increasing their susceptibility to

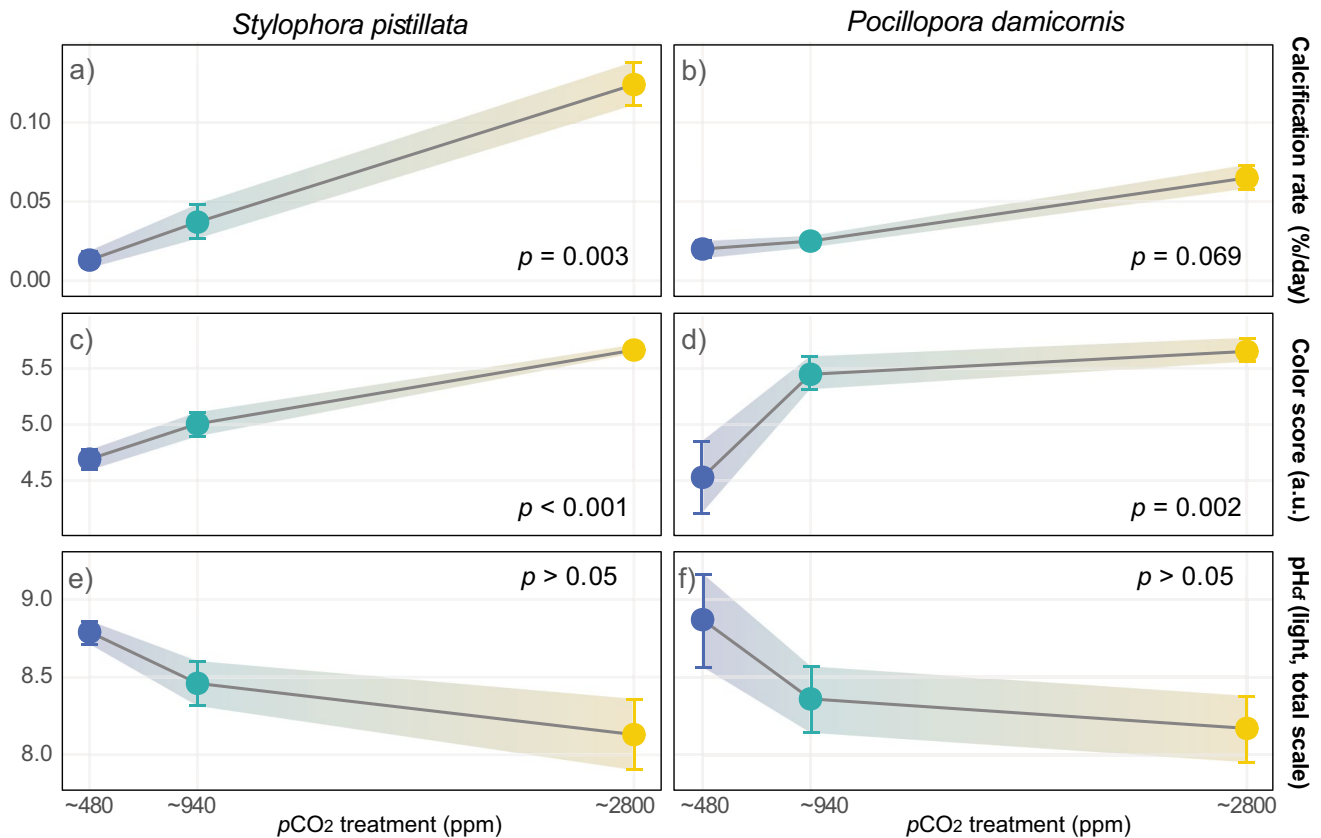


Fig. 1 Responses of **a, c, e** *Stylophora pistillata* and **b, d, f** *Pocillopora damicornis* to increasing $p\text{CO}_2$ at 28 °C replotted for context. Points show treatment means $\pm$ SE computed from reported data. **a, b** Calcification rates (% dry wt day⁻¹) and **c, d** color score are from

Cameron et al. (2022), **e, f** calcifying fluid pH (pH_{CF} , light) is from Guillermic et al. (2021). Global p -values for the $p\text{CO}_2$ effect (from the original studies) are shown in facets

OA. This study builds on published physiological data (Fig. 1; Guillermic et al. 2021; Cameron et al. 2022) and uses comparative LC–MS/MS-based proteomics from the same experimental coral specimens to investigate effects of moderate (~940 ppm) and high (~2,800 ppm) $p\text{CO}_2$ treatments compared to controls (~480 ppm) over two months.

Given observed differences in OA resistance between species, we anticipated *S. pistillata* may exhibit a more dynamic proteomic response, involving increased abundance of proteins related to ion regulation, calcification, and energy metabolism. *P. damicornis* was expected to show a more constrained response, focusing on stress defense. Due to host–symbiont interaction complexity and limited proteomic data under long-term OA exposure, our approach aims to detect patterns in protein abundance changes and identify pathways involved in coral acclimatization to high seawater $p\text{CO}_2$. Our results elucidate species-specific coral resistance and vulnerability to future ocean conditions, stimulate future studies and inform reef management.

Materials and Methods

Coral Selection and Experimental Design

The experiment focused on two reef-building coral species, *Pocillopora damicornis* and *Stylophora pistillata*, both of which are widespread across tropical reefs. Its design and monitoring of seawater parameters have been described in detail previously (Guillermic et al. 2021; Stühr et al. 2021; Cameron et al. 2022). Coral fragments were originally sourced from various colonies in the Fiji Islands. Species identification followed the distributor's classification since no genetic material was preserved, and we acknowledge that the colonies may represent closely related lineage within the species complex. Pocilloporids generally harbor dinoflagellate endosymbionts from the Symbiodiniaceae family, with both corals mostly containing *Cladocopium goreaui* and *Fugacium kawagutii*.

To simulate ocean acidification scenarios, corals were exposed to three $p\text{CO}_2$ conditions: ~480 ppm (control; slightly above the current global average atmospheric

CO₂ concentration), ~940 ppm (moderate; year 2100 under high-emission scenarios (IPCC 2022)), and ~2,800 ppm (high; beyond year 2300 under persistent high-emission trajectories (Kawahata et al. 2019), an extreme level included to elicit strong physiological responses and assess mechanistic limits) over a period of eight weeks, following a 3-week acclimation phase. Experiments were conducted under controlled temperature conditions (28 °C) in a flow-through seawater system with four replicate tanks per treatment. The experiments were carried out in the marine experimental facility (MAREE) at the Leibniz Centre for Tropical Marine Research (ZMT) in Bremen, Germany, and also included a set of higher temperature treatments. While we here focus on the *p*CO₂ treatments at control temperature only, proteomic data obtained for the effects of thermal stress (31 °C) at high *p*CO₂ on *S. pistillata* are accessible though the data repository.

Briefly, coral fragments were affixed to eggcrate stands labeled with unique identifiers and evenly distributed across experimental treatments and replicate tanks, ensuring equivalent size ranges. Corals were cultured in 10-L replicate acrylic tanks, with seawater supplied by 244-L recirculating sumps equipped with mechanical filters, protein skimmers, and activated charcoal filters. To maintain stable conditions, natural seawater sourced from Spitsbergen, Norway, was continuously added to the system at a rate of 0.6 L per hour, preventing depletion of alkalinity, [Ca²⁺], and other seawater constituents throughout the study. Lighting conditions were regulated at 150 lx using actinic blue and white lights on a 12-h cycle. Nutrition was sustained by feeding *Artemia salina* nauplii at the end of every other day, with all filtration material cleaned the next morning. Corals were acclimated by initially maintaining them under control conditions for the first week, followed by gradual exposure to increasing *p*CO₂ until reaching target conditions in the second week, which were then maintained for another week.

Sample collection, protein extraction and LC–MS/MS analysis

All fragments were collected after 62 days of treatment exposure. The corals were briefly rinsed with cooled, sterilized seawater, and the tissue was transferred from the skeleton into plastic zip-lock bags using an airbrush. The tissue slurries were immediately snap-frozen and stored at -80°C until processing. To gain insight into the underlying cellular responses of corals, a label-free quantitative proteomics approach based on liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) was applied, adjusted for coral samples from established methods (Stuhr et al. 2018, 2021).

Proteomic data processing and analysis

Detailed descriptions of proteomic analyses and data processing are available in the supplementary materials. Briefly, proteins were extracted using a 5% SDS buffer containing 50 mM Tris, 150 mM NaCl, and protease inhibitors to prevent protein degradation. Coral tissues were homogenized via mechanical grinding and ultrasonication to shear nucleic acids. Lysates were centrifuged and supernatants collected. Protein concentrations were determined using bicinchoninic acid (BCA) assay, and equal amounts were used for the downstream digestion. Disulfide bonds were then reduced and alkylated using dithiothreitol (DTT, 10 mM; incubation at 56 °C for 30 min) and iodoacetamide (IAA, 30 mM; incubation at room temperature in dark for 30 min), respectively. Proteins were precipitated with cold ethanol, washed with acetone, and resuspended in trypsin digestion buffer. Digestion was performed overnight using sequencing-grade trypsin. For nano-LC–MS/MS analysis (Ultimate 3000 RSLC, Thermo Q Exactive HF), desalted peptides were resuspended in 0.1% TFA, and 400 ng of digested peptides per sample was injected onto the column. Peptides were trapped (2 cm × 100 µm C18) and separated (50 cm × 75 µm C18) over a 90 min gradient. Data were acquired in data-dependent mode, fragmenting the top 15 precursors via HCD (higher-energy collisional dissociation). Mass spectrometry proteomics data have been deposited in the ProteomeXchange Consortium via the PRIDE (Vizcaíno et al. 2016) partner repository (dataset identifier PXD062918).

Raw data were analyzed in Proteome Discoverer v1.4 using Mascot and Sequest, searching against species-specific coral–Symbiodiniaceae databases (Voolstra et al. 2017; Cunning et al. 2018). Peptide-spectrum matches were filtered at 1% FDR (false discovery rate), requiring ≥ 1 unique peptide and ≥ 2 PSMs (peptide-spectrum matches). These metrics are contained in the deposited raw data and were used to compute normalized spectral abundance factors (NSAF), which correct for peptide number and protein size in the quantitative analyses (Zybailov et al. 2006). NSAF values were used to quantify protein abundance and log(x + 1)-transformed for statistical analysis in R v.4.3.2 (R Core Team 2016). Symbiodiniaceae-associated proteins were excluded for both species from further analyses and discussion. Differentially abundant proteins (DAPs) between treatments (with n = 3 replicates each) were identified using generalized linear models (adonis function, R package 'vegan'), with significance set at FDR-adjusted *p* ≤ 0.05 (Benjamini–Hochberg correction). For data normalization, scaling factors were applied assuming most proteins remain stable across treatments. Non-detects were imputed using robust regression on order statistics (R package 'NADA'), assuming a log-normal distribution. Proteins with > 33%

non-detects were excluded. Functional annotation was performed with eggNOG-mapper (Huerta-Cepas et al. 2019) and UniProtKB via BLAST. Principal component analyses and volcano plots ('ggplot2,' 'ggrepel') were used to visualize treatment effects. PCAs were conducted on all DAPs to visualize proteomic differences between samples, using unit variance scaling for rows and singular value decomposition. To focus on the most strongly regulated proteins, we extracted DAPs with $\log_2\text{FC} \geq \pm 1$ and post hoc $p \leq 0.05$ (relative to controls), excluding those that differed only between the moderate and high $p\text{CO}_2$ treatments. The resulting differentially concentrated proteins (DCPs) were grouped by functional categories based on GO annotations. Heatmaps with hierarchical clustering (average linkage, correlation distance) were generated using treatment means of log-transformed NSAF values. For *S. pistillata* under high $p\text{CO}_2$, the top regulated proteins were identified based on a combined rank of fold-change and p -value.

Results

Protein identification and association

In total, 6,631 and 4,576 proteins were identified for *S. pistillata* (5,392 host coral-, 709 SymbC1- and 530 SymbF-associated; Table S2) and *P. damicornis* (3,486 host coral-, 789 SymbC1- and 301 SymbF-associated; Table S4) homogenates, respectively. Per sample, a total of 1,030–3,109 host proteins were identified, corresponding to an average sequence coverage of 8–13% and 3–4 unique peptides per

protein (Table S1). Average peptide length ranged from 580 to 700 amino acids across treatments. After removing low-abundance protein clusters, 4,903 proteins remained for *S. pistillata* and 4,085 for *P. damicornis*, of which 1,674 and 1,234 were commonly found across all treatments. Since our extraction protocol was designed to mainly lyse the cnidarian host cells, the majority of identified proteins were coral-associated (see sect. "Proteomic approach and methodological considerations"). Symbiont-associated proteins were thus removed from downstream analyses and interpretation.

Patterns of differentially abundant proteins

Generalized linear models identified significant changes in abundance across treatments ($p \leq 0.05$) for 315 differentially abundant proteins (DAPs) in *S. pistillata* and 55 DAPs within *P. damicornis*. These represent 19.5 and 5% of the cleaned host proteomes detected, respectively, showing that fewer proteins were affected by elevated $p\text{CO}_2$ -treatment in *P. damicornis* than in *S. pistillata* (Tables S1–S2), especially at high $p\text{CO}_2$ conditions, revealing distinct proteomic changes.

Multivariate analyses of all DAPs of each species confirmed strong reproducibility, with biological replicates clustering tightly within treatments in principal component analyses (Fig. 2). When the PCA was extended to all quantified proteins (Fig. S1), only *S. pistillata* showed a partial treatment separation, whereas *P. damicornis* samples largely overlapped. This pattern indicates that the stronger OA response of *S. pistillata* involved broader proteome-wide

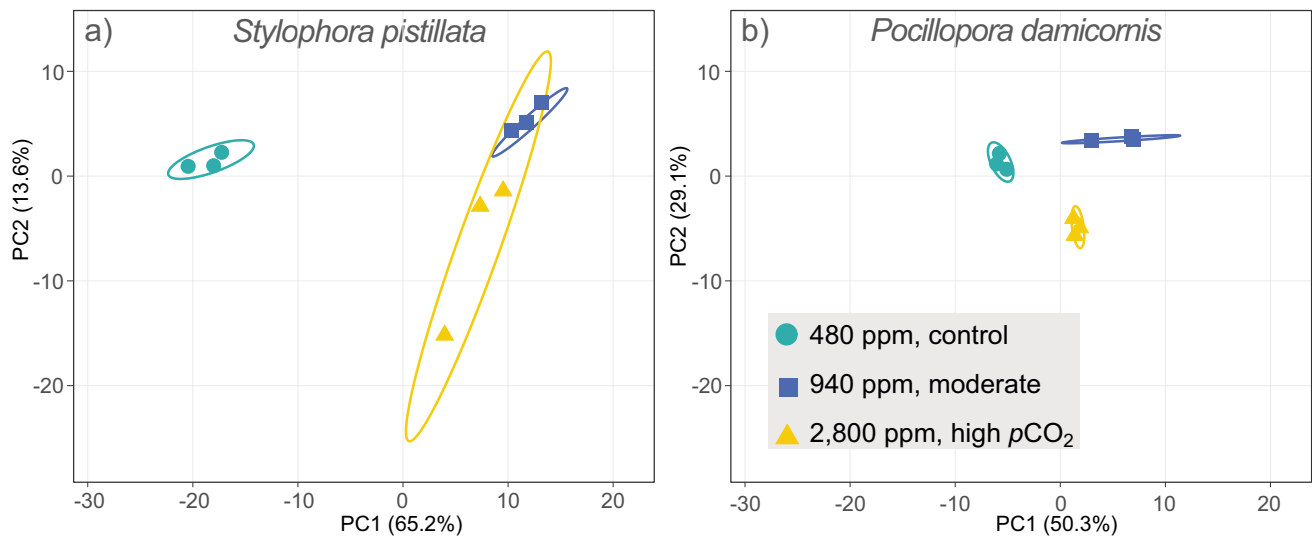


Fig. 2 Principal component analyses and volcano plots of all proteins that were significantly different in abundance between treatments (GLM $p \leq 0.05$) of **a** *Stylophora pistillata* and **b** *Pocillopora damicornis*. PCAs are based on unit variance scaled log-transformed

NSAF values. Principal components (PC) 1 and 2 collectively explain ~80% of the total variance in both species. Prediction ellipses are such that with probability 0.95, a new observation from the same group will fall inside the ellipse

shifts, while changes in *P. damicornis* were confined to a smaller subset of proteins.

Volcano plots (Figs. 3, S1, S3) revealed species-specific responses to ocean acidification, highlighting the number and direction of regulated proteins under moderate and high $p\text{CO}_2$ treatments relative to control conditions. These patterns show a stronger and more directional proteomic response to elevated $p\text{CO}_2$ in *S. pistillata*, while *P. damicornis* responded more modestly and variably across treatments.

Changes of protein abundances across treatments

To focus further on the most reliably identified proteins (identified by ≥ 1 unique peptides) and only those that showed the most substantial increases or decreases compared to controls, we excluded all DAPs with $\log_2\text{FC} < 1$, the symbiont-associated proteins as well as those that were only significantly different between high and moderate $p\text{CO}_2$ treatments from further analysis and discussion. This resulted in 219 differentially concentrated proteins (DCPs) for *S. pistillata* and only 33 remaining DCPs for *P. damicornis* (colored protein in Figs. 3 and 4).

For *S. pistillata*, ten proteins were decreased and three were increased in response to moderate OA, while high OA elicited a much stronger response, with 131 proteins decreasing and 85 increasing in abundance compared to controls (Figs. 4, 5 and Table S3). Interestingly, ten proteins exhibited overlapping responses across both OA levels, with one decreased and nine increased in quantity, highlighting a core set of proteins consistently involved in OA acclimatization.

In *P. damicornis*, moderate OA resulted in six decreased and 12 increased proteins, whereas high OA caused nine proteins to decrease and 15 to increase in abundance (Figs. 4, 6 and Table S5). Notably, nine proteins exhibited consistent changes across both OA levels, including three decreased and six increased proteins, demonstrating a shared proteomic basis for responses at different levels of acidification.

The functional gene ontology (GO) analysis revealed species-specific patterns of protein quantity changes under both elevated $p\text{CO}_2$ conditions, evident in the distribution patterns of functional groups assigned to DCPs (Figs. 3, 5, 6 and S2). Their functional implications are outlined in the SI and discussed below.

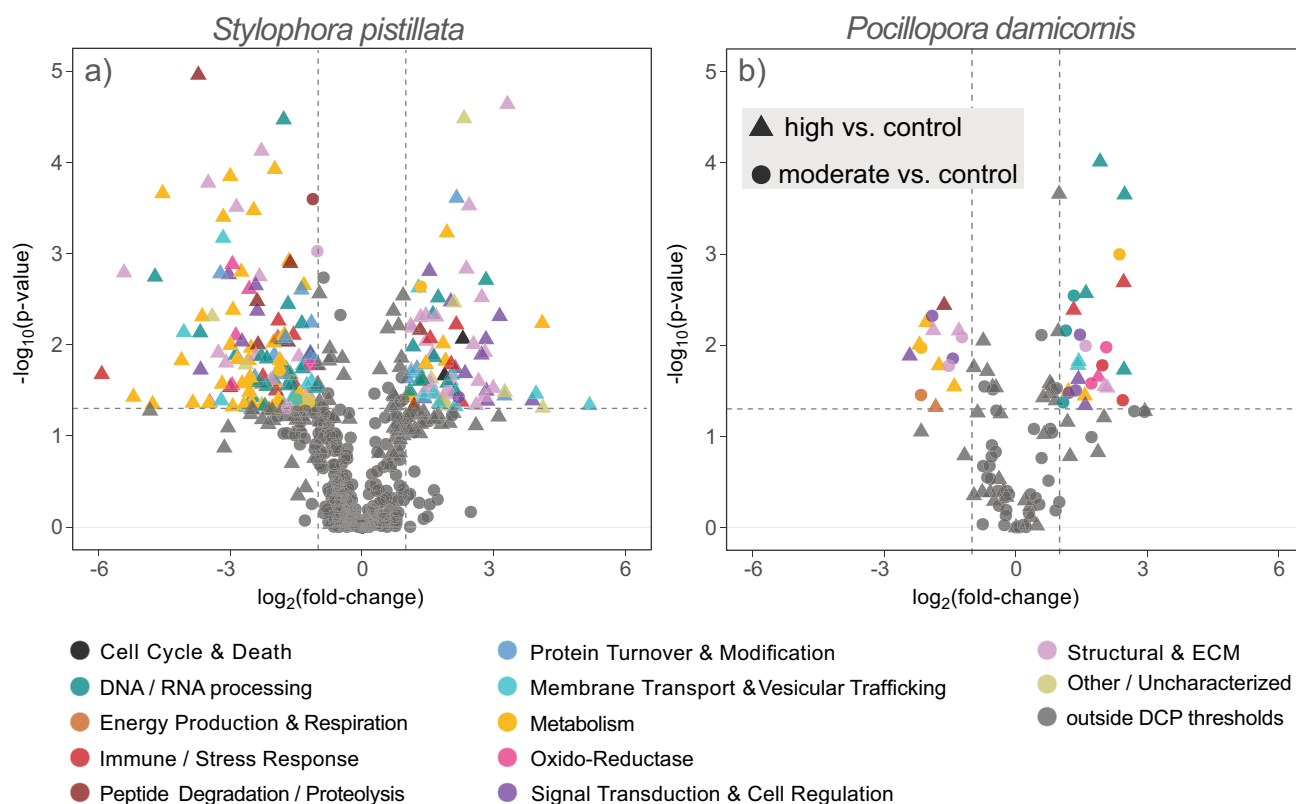
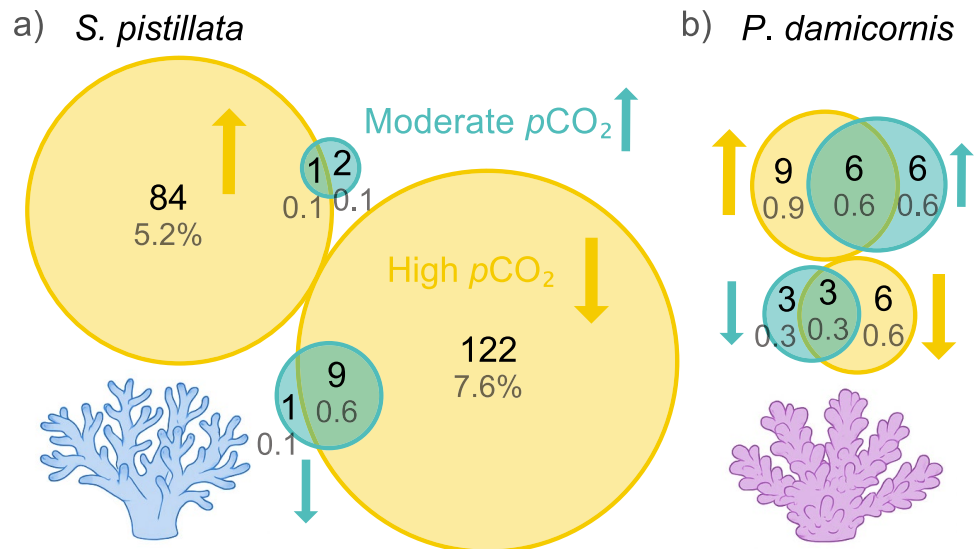


Fig. 3 Volcano plots show of all differentially abundant proteins at high or moderate $p\text{CO}_2$ compared to controls with functional grouping of differentially concentrated proteins (DCPs) indicated by colors. Dashed lines at $\log_2\text{FC} = \pm 1$ (vertical) and at $p = 0.05$ (horizontal)

highlight the thresholds for significance to be considered as DCPs, with proteins on the left being depleted and proteins on the right being elevated; respective protein descriptions are shown in Figs. S2 and S4

Fig. 4 Venn diagrams showing numbers of DCPs in **a** *Stylophora pistillata* and **b** *Pocillopora damicornis* at moderate and high $p\text{CO}_2$ compared to controls; respective percentages (%) of all identified proteins common across treatments below; increases and decreases indicated by arrows



Discussion

Proteomic approach and methodological considerations

We applied a previously developed high-throughput proteomics approach for reef-associated holobionts (Stuhr et al. 2018, 2021) to study responses of two tropical scleractinian coral species under ocean acidification scenarios. The protocol was adapted for tissue availability, with quality control steps ensuring robustness. However, the limited symbiont protein identification in both corals and across all treatments indicates that Symbiodiniaceae cell lysis within coral homogenates may have been insufficient due to the resistant dinoflagellate cell walls. Although other studies also reported low proportions of annotated symbiont proteins (Lin et al. 2022) or muted symbiont gene expression responses *in hospite* (Jiang et al. 2022), we decided to exclude the response of the Symbiodiniaceae proteome in our study and recommend future studies improve host-symbiont separation through centrifugation and stronger dinoflagellate cell lysis methods.

Protein identification rates were slightly higher in *S. pistillata* than *P. damicornis* (5,392 vs 3,486 host proteins). This difference may result from genome annotation coverage differences (Voolstra et al. 2017; Cunning et al. 2018). Yet, after quality control, symbiont-protein removal and filtering for those that were common across all treatments, 1,614 and 1,013 proteins remained, respectively. *S. pistillata* exhibited a substantially stronger proteomic response to elevated $p\text{CO}_2$ (Figs. 2a, 3a, 4), with more than sixfold more differentially concentrated proteins (DCPs) than *P. damicornis* (Figs. 2b, 3b, 4), which indicate distinct species-specific strategies under severe OA conditions. The broad response of *S. pistillata* is also evident at the global level (Fig. S1), where it

shows separation of the high $p\text{CO}_2$ treatment, indicative for a more systemic, coordinated proteomic shifts, whereas *P. damicornis* exhibited limited and more variable adjustments.

We consequently compare both species' data and put it into the context of other molecular studies on OA effects on coral. However, comparing OA experiments from the literature is challenging due to different host-symbiont associations, setups, and methodologies (Wahl et al. 2016). Many immediate gene expression responses to OA are transient (Moya et al. 2015; Rocker et al. 2015), while proteomic changes offer more stable acclimation (Diz et al. 2012). Unfortunately, mainly transcriptomic studies are available for comparison while proteomic analyses of coral responses to ocean acidification remain rare, limiting direct molecular comparisons across studies. Where relevant, we therefore reference gene expression studies to illustrate general regulatory trends and to highlight which of these predicted processes are supported (or not) by our proteomic evidence.

Proteomics reveals species-specific metabolic strategies under OA

Overall, the effects of acidification on coral health are dose-dependent and species-specific. Both species in this experiment showed increased growth rates (measured via buoyant weight changes) with increasing $p\text{CO}_2$ (Guillermic et al. 2021; Cameron et al. 2022) and higher photosymbiont or photopigment densities (Fig. 1). This continuous enhanced growth was attributed to elevated CO_2 fertilizing Symbiodiniaceae photosynthesis (Ries et al. 2009; Herrera et al. 2021; Helgoe et al. 2024). Enhanced dissolved inorganic carbon (DIC) fixation and photosynthate production may benefit the coral host through increased energy supply, enabling stronger proton-pumping activity and elevated pH_{CF} ,

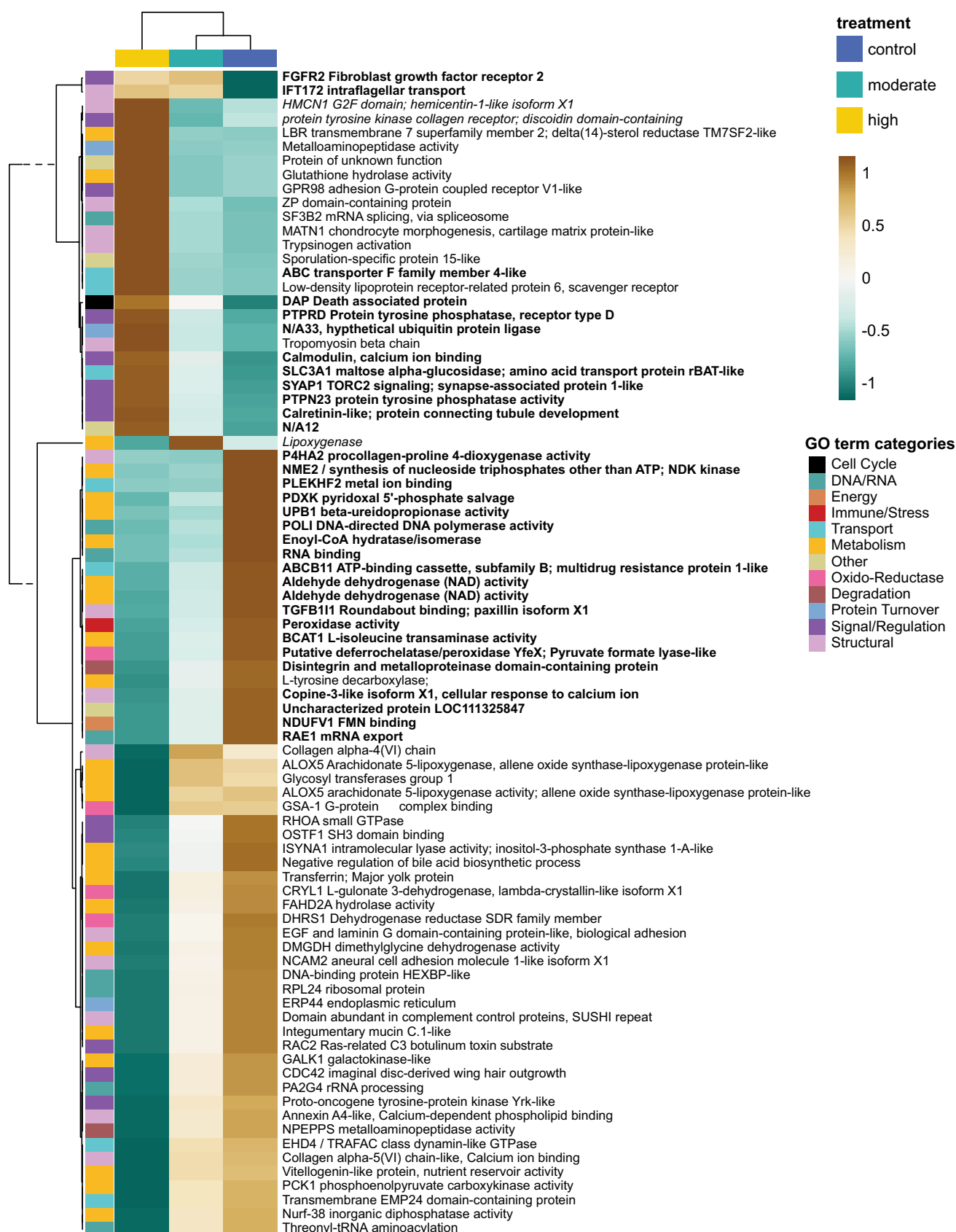


Fig. 5 Heatmap of all differentially concentrated proteins at moderate (~940 ppm; n=35) and the top 60 DCPs at high (~2,800 ppm; n=60) $p\text{CO}_2$ levels compared to controls (~480 ppm; GLM, $p \leq 0.05$ and $\log_2\text{FC} \geq \pm 1$) in *Stylophora pistillata*, with functional grouping indicated by colors and core proteins changed in quantity in both treatments in bold or italics (if contrasting). Dendrograms indicate cluster analyses of treatments and DCPs using correlation distance and average linkage, based on log-transformed mean normalized spectral abundance factor (NSAF) values. Rows are centered by unit variance scaling; protein names and short descriptions are included. The full heatmap of all DCPs is included in appendices as Fig. S3, with detailed descriptions in Table S3

supporting calcification. While both species increased proton regulation of their calcifying fluid, i.e., the difference between seawater and calcifying fluid proton concentrations $\Delta[\text{H}^+]$ as measured by microelectrodes with increasing $p\text{CO}_2$ (Guillermic et al. 2021; Cameron et al. 2022), *S. pistillata* showed an over twofold higher growth compared to *P. damicornis*. This implies greater biological control over pH_{CF} , causing higher energetic demand in *S. pistillata* under OA conditions. *S. pistillata* in other studies maintained better pH of the extracellular calcifying fluid under acidification, compared to *P. damicornis* (Venn et al. 2019).

Ocean acidification triggered distinct proteome responses between *S. pistillata* and *P. damicornis*, highlighting different metabolic strategies despite close phylogenetic relationship. *S. pistillata* showed more differentially concentrated proteins under elevated $p\text{CO}_2$ compared to controls (DCPs), indicating extensive metabolic adjustments that may reflect both active reprogramming and heightened cellular stress while *P. damicornis* exhibited fewer changes (Fig. 4). These responses align with previous observations on these corals (Guillermic et al. 2021; Cameron et al. 2022) and reveal differences in each species' capacity for physiological acclimatization. Remarkably, the moderate $p\text{CO}_2$ treatment (~940 ppm) caused only limited proteomic adjustments in both species (Fig. 4, Tables S1–S2) with few consistent directional changes, which shows that cellular homeostasis was largely maintained under near-future acidification levels. Such resilience may reflect efficient physiological buffering or the capacity of these corals to tolerate incremental decreases in seawater pH.

For *S. pistillata* (Figs. 5, S2–S3, Tables S1–S2), the proteome showed depletion of enzymes linked to carbon and lipid metabolism (ALDH1L2, PCK1, SELENBP1, MYP), amino acid turnover (BCAT1, UPB1), and cofactor metabolism (PDXK), as well as small molecule and carboxylic acid catabolism (BPHL, FAHD2A), suggesting broad metabolic suppression for energy conservation. The species increased multiple ion transporters and some stress-related proteins (ATP1B1, STOM, SLC9A3R1, SLC25A11, cell death-inducing and heat shock factors), implicating a shift toward supporting calcification fluid chemistry and cellular defense. Extracellular matrix (ECM) components (HMCN1, MATN1,

laminin, collagen isoforms) were differentially abundant, indicating selective remodeling of the organic matrix.

By contrast, *P. damicornis* displayed fewer proteomic changes (Figs. 6, S4, Tables S4–S5), with metabolic proteins ALDH1L2, FBPI, CRAT, and ASNS slightly decreased, but the absence of broad suppression suggests a conserved metabolic baseline. Notably, some antioxidant and redox-related proteins (TXNDC17, MOXD1, SELENBP1) increased, showing enhanced oxidative stress response. Elevated troponomyosin, hemicentin-1, CHL1, NCL1, and calbindin-32 indicate cytoskeletal and cell adhesion adaptations, while modest changes in signaling proteins (EIF3A, GIGYF2, GLIPR2, ADRBK2) suggest subtle intracellular communication responses. This indicates a measured approach to sustaining energy metabolism and structural integrity, potentially reflecting lower energetic demands or an inherently efficient stress response strategy. Membrane transport components ATP2A2, SLC8A1, and NSF vesicle-fusing ATPase showed varied trends, hinting toward nuanced alterations in ion homeostasis.

***Stylophora pistillata*: Intense metabolic reallocation and energetic trade-offs**

In *S. pistillata*, a high overall number of DCPs emerged with increasing $p\text{CO}_2$ —especially under the high $p\text{CO}_2$ treatment, where the DCP count exceeded that at moderate $p\text{CO}_2$ by nearly 16-fold. This upsurge in regulated proteins underscores strong cellular support for physiological plasticity and metabolic adjustments under OA. The broad reduction of proteins associated with energy metabolism, including BPHL, PDXK, BCAT1, UPB1, and FAHD2A, indicates suppression of vitamin B6 metabolism, amino acid degradation, and oxidation of small organic molecules. Such metabolic suppression is a hallmark of stress mitigation in corals, including seawater acidification (Kaniewska et al. 2012; Moya et al. 2012; Putnam et al. 2016; Glazier et al. 2020; Scucchia et al. 2021), allowing energy reallocation for cellular maintenance. While differential expression of lipid metabolism genes was reported in other experiments (Moya et al. 2012) and CO_2 -seep adapted corals (Kenkel et al. 2018), increased feeding rates and lipid storages in *Acropora cervicornis*, *Cladocora caespitosa* and *Astroides calycularis* (Towle et al. 2015; Hulver et al. 2024) likewise indicate behavioral and metabolic plasticity as key OA responses.

S. pistillata's response to high $p\text{CO}_2$ featured increased ion regulatory proteins for intracellular homeostasis and pH regulation, contrasting these metabolic cutbacks. Na^+/K^+ -transporting ATPases, often localized to the calicoblastic epithelium support electrochemical gradients facilitating proton extrusion (Zoccola et al. 2015; Barott et al. 2020; Capasso et al. 2021). The observed upsurge of ATP1B1

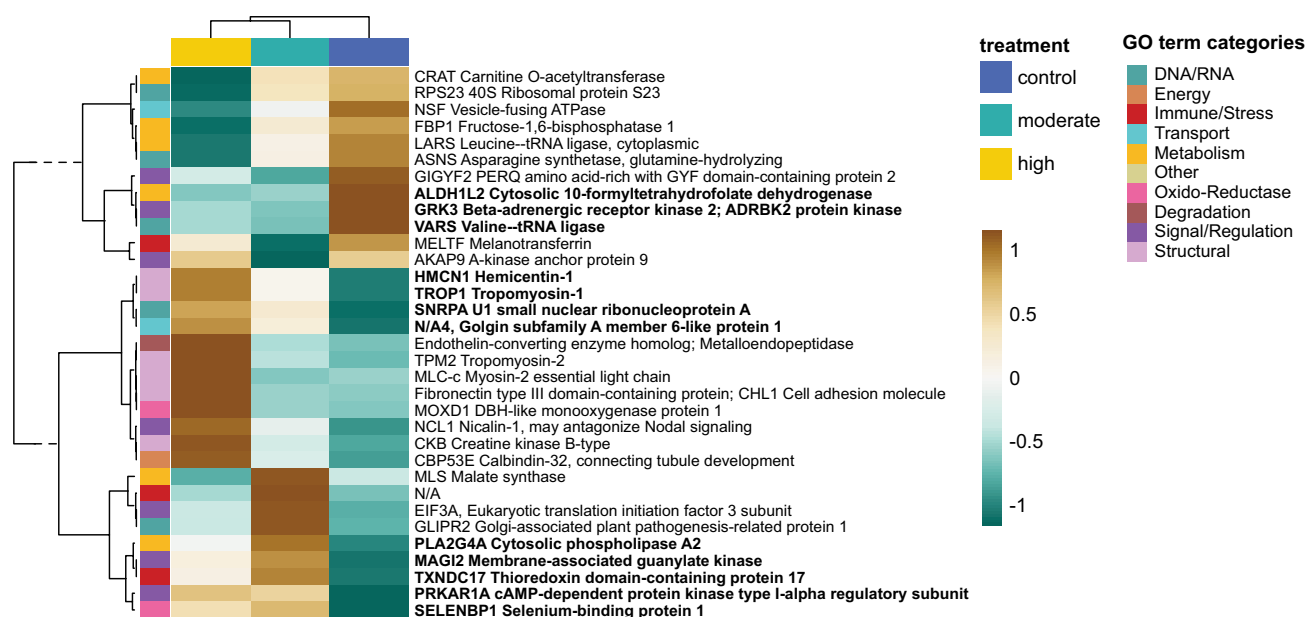


Fig. 6 Heatmap of all differentially concentrated proteins ($n=33$) at moderate (~ 940 ppm) or high ($\sim 2,800$ ppm) $p\text{CO}_2$ levels compared to controls (~ 480 ppm; GLM, $p \leq 0.05$ and $\log_2\text{FC} \geq 1$) in *Pocillopora damicornis*, with functional grouping indicated by colors and core proteins changed in quantity in both treatments in bold or italics

(if contrasting). Dendrograms indicate cluster analyses of treatments and DCPs using correlation distance and average linkage, based on log-transformed mean normalized spectral abundance factor (NSAF) values. Rows are centered by unit variance scaling; protein names and short descriptions are included, with detailed descriptions in Table S5

suggests its involvement. The increase of SLC9A3R1, a scaffold protein regulating Na^+/H^+ exchangers, indicates enhanced acid–base regulation at the tissue–skeleton interface, as previously observed by increased other SLC9 family member expression at reduced pH (Capasso et al. 2021). STOM modulates acid-sensing ion channels, similar to other acid–base sensing enzymes localized in coral calcifying cells, helping elevate pH at calcification sites (Barott et al. 2020). Collectively, these may maintain proton gradients essential for calcification (Vidal-Dupiol et al. 2013; Zoccola et al. 2015; Davies et al. 2016). This aligns with studies on pH regulation in biomineralization under OA (Venn et al. 2019). The abundance of ABC transporter F family member 4-like, LDL receptor-related protein 6 and mitochondrial carrier protein SLC25A11 were also highly elevated, possibly serving in ion, energy and nutrient transport and signaling roles. While the expression and methylation state of such genes was reduced under long-term low pH (Liew et al. 2018), SLC48 and ABCG4 increased in the proteome of *Galaxea fascicularis* after 10 days (Lin et al. 2022). Along with our proteomic data, this emphasize that transcript-level changes may not necessarily translate into lower protein abundance.

Marked elevation of cytoskeletal proteins under high $p\text{CO}_2$ (myosin, spectrin, calmodulin) showed support of vesicle trafficking and intracellular delivery of calcification-related materials (Mass et al. 2016; Takeuchi et al. 2016). Concurrently, organic matrix proteins involved in

mineral scaffolding (tropomyosins, calbindin, ZP-domain proteins) increased, while others (von Willebrand factor type A, annexin A4-like protein, collagen and laminin isoforms) were decreased. Selective remodeling of the extracellular OM may modulate nucleation sites, crystal orientation, and skeletal morphogenesis under OA (Peled et al. 2020; Zaquin et al. 2022). Observed proteomic changes support mechanisms by which corals maintain structural skeleton organization despite altered saturation states (Mass et al. 2016; Leiva et al. 2023), supporting findings that organic molecules can modify CaCO_3 polymorphs for a structural rather than catalytic role in calcification (Hohn and Reymond 2019). Cytoskeletal and scaffolding proteins often respond under stress (Maor-Landaw and Levy 2016), demonstrating cytoskeletal framework reinforcement. Likewise, *S. pistillata* under OA for ~ 2 years strongly increased expression of proteins providing structural support within the ECM (Liew et al. 2018).

In tandem, *S. pistillata* boosted levels of stress response proteins, including glutathione and quinone oxidoreductases (GST, QOR), heat shock factors, and chaperones, while other antioxidants such as catalase declined. Stress response markers (HSPs, HSFs) were likewise overexpressed in juvenile *A. millepora* (Lin et al. 2022), along with Bcl-2 genes, peptidases and caspases, indicating apoptosis suppression at elevated $p\text{CO}_2$ to be an acclimation mechanism (Moya et al. 2015).

Finally, despite the observed depression in host metabolic pathways, *S. pistillata* showed significantly increased Symbiodiniaceae densities, i.e., a higher color-based photosymbiont index (Fig. 1; Cameron et al. 2022). This supports the idea that the enhanced symbiont populations may help compensate the coral's energy budget under high $p\text{CO}_2$ condition by sustaining the translocation of photosynthates to the host (Tremblay et al. 2013; Gibbin and Davy 2014). This aligns with previous observations of other resilient corals that exhibit increased investment in cellular maintenance and calcification-related processes under acidification stress (Scucchia et al. 2021; Jiang et al. 2022). Overall, *S. pistillata* under OA presents a high-energy, plastic physiological response that may sustain calcification and growth despite increased metabolic costs, potentially balanced by enhanced symbiont density.

***Pocillopora damicornis*: Moderate adjustments indicating balanced response**

By contrast, *P. damicornis* displayed fewer DCPs across both moderate and high $p\text{CO}_2$ conditions, with higher inter-individual variation in protein abundances (Fig. 3). Rather than a strong metabolic shutdown, its proteomic profile hints at a more balanced approach to OA stress. Core metabolic enzymes including ALDH1L2, FBPI1, CRAT, and ASNS were slightly decreased, indicating mild suppression of energy metabolism. This suggests *P. damicornis* maintained stable baseline metabolism by mobilizing energy reserves to meet physiological demands as previously suggested for *P. damicornis* and other pocilloporids at low pH due to upregulation of autotrophic and heterotrophic related genes (Vidal-Dupiol et al. 2013; Scucchia et al. 2021).

Moreover, some signaling, DNA/RNA processing, and cell adhesion proteins decreased (e.g., 40S ribosomal protein S23, Leucine- and Valine-tRNA-ligases LARS and VARS, an insulin-like growth factor GIGYF2, and ADRBK2). Their response resembles *S. siderea*, which responded parabolically to severe OA treatments (Castillo et al. 2014), upregulating metabolic pathways and specific transporters (Davies et al. 2016) to sustain calcification, but probably reaching adaptive limits at extreme $p\text{CO}_2$ levels.

Cytoskeletal and structural proteins like tropomyosin, calbindin, HMCN1, CHL1, and NCL1 were moderately increased, indicating subtle cellular remodeling. The elevation of MYL6 may indicate changes in actin-myosin dynamics relevant to both contraction and intracellular transport. Interestingly, MYL6 abundance diverges from its depression in the *G. fascicularis* proteome under short-term low pH (Lin et al. 2022), suggesting time- or species-specific roles.

Unlike *S. pistillata*, more antioxidant and redox-related proteins, such as TXNDC17, MOXD1, and SELENBP1, were increased, showing enhanced oxidative stress

management in *P. damicornis*. Acidification can lead to accumulation of reactive oxygen species (ROS), damaging lipids and membranes, triggering anticipatory or homeostatic antioxidant responses through upregulation of detoxification pathways to enhance tolerance (Maor-Landaw and Levy 2016).

As in *S. pistillata*, only few membrane transport components (Golgin subfamily A member 6-like protein 1, sarco-plasmic/endoplasmic reticulum Ca^{2+} -ATPase ATP2A2, $\text{Na}^+/\text{Ca}^{2+}$ exchanger SLC8A1, and NSF vesicle-fusing ATPase) changed without consistent directionality, possibly reflecting localized regulation. Two stimulus response receptor-type proteins decreased in *P. damicornis*, indicating immune response strategies linked to long-term acidification adaptation (Kenkel et al. 2018; Leiva et al. 2023). These patterns indicate a balanced strategy—enough metabolic adjustment to protect essential functions, without the extensive reallocation seen in *S. pistillata*.

From proteomic shifts to physiological resilience

In both species, maintained calcification under elevated $p\text{CO}_2$ highlights the importance of well-orchestrated acid–base regulation and photosymbiont contributions, supporting findings that stable calcification under high OA relates to increased biological control of calcifying fluid chemistry (Ries et al. 2009; Leung et al. 2022). Yet, the integration of proteomic changes with measured physiological outcomes shows differential resilience strategies in *S. pistillata* and *P. damicornis* under OA, diverging in magnitude and function.

S. pistillata showed a shift in energy allocation, investing in ion transport (ATP1B1, SLC9A3R1), cytoskeletal reorganization (tropomyosin, calmodulin), and ECM synthesis (HMCN1, MATN1, collagen), with a drop in general metabolism (PDXK, UPB1, BCAT1). These data support findings that *S. pistillata* increases physiological performance (i.e., growth and calcification) under high $p\text{CO}_2$ by investing in homeostatic regulation (Guillermic et al. 2021), a pattern consistent with elevated internal pH_{CF} under OA (Venn et al. 2019; Cameron et al. 2022). This requires metabolic cost, likely offset by increased photosymbiont density or heterotrophic feeding, which increases under elevated $p\text{CO}_2$ (Towle et al. 2015; Hulver et al. 2024).

In contrast, *P. damicornis* adopted a more moderate strategy, maintaining metabolic function with limited systemic suppression of energy-producing and ion-regulating processes. While moderate adjustments in ion transport (ATP2A2, NSF) and oxidative stress proteins (TXNDC17, MOXD1) were evident, no widespread proteomic reprogramming occurred. This aligns with a strategy focused on physiological stability over flexibility, supporting observations of more conservative phenotypic plasticity in

this species (Vidal-Dupiol et al. 2013; Jiang et al. 2022). Venn et al. (2019) reported that the pH regulation of ECM and calicoblastic cells declined more in *P. damicornis* than *S. pistillata* under OA treatment, in line with our proteomic results.

The integration of molecular and physiological data reveals how divergent proteomic strategies align with different modes of resilience: a metabolically plastic, energetically demanding strategy in *S. pistillata* versus a balanced, potentially less flexible approach in *P. damicornis*. These align with responses under thermal stress (Guillermic et al. 2021; Cameron et al. 2022), where *P. damicornis* showed decreasing pH_{CF} and skeletal dissolution under all OA treatments, while *S. pistillata* did not. *P. damicornis* also exhibited more intense bleaching under thermal stress, indicating overall lower resilience to climate change. *S. pistillata*'s stronger proteomic response explains its maintained physiological function despite OA stress, while *P. damicornis*' moderate proteomic shifts may have been insufficient to effectively counteract stressors. These strategies help explain species-specific OA tolerance differences across reef-building corals (Hoadley et al. 2015; Putnam et al. 2016), highlighting the importance of integrating cellular-level responses (e.g., proteomic) with whole-organism outcomes (e.g., calcification, bleaching) to better predict species-specific vulnerabilities under future climate change scenarios.

Biological control of calcification and ecological implications

The proteomic data strongly support that biological control of calcification plays a critical role in coral responses to OA. Both species showed quantitative changes of key proteins involved in pH homeostasis, ion transport, and ECM structure, consistent with maintaining favorable calcifying conditions via active cellular processes being essential under low pH (Ries 2011; Zoccola et al. 2015; Venn et al. 2019). In *S. pistillata*, the increase of SLC9A3R1, SLC25A11, and ATP1B1 suggests enhancement of proton and ion transport capacity, potentially contributing to acid–base regulation in calcifying or intracellular compartments, consistent with biological control of calcifying fluid pH, although a contribution from symbiosome or lysosomal acidification cannot be excluded. In contrast, several other ion transport and trafficking proteins (ATP1B1, NSF, Copine-3-like protein, EHD4/TRAFAC class dynamin-like GTPase) were depleted. Depression of proton-channel expression, but increases of sodium–potassium transport, cell membrane receptors and ABC transporters appear common in cnidarian cells under OA (Kaniewska et al. 2012; Maor-Landaw and Levy 2016; Lin et al. 2022), while increased transcription of ECM proteins (laminin, collagen, MATN1, HMCN1) may support matrix-mediated nucleation control and crystal growth

regulation under altered conditions (Mass et al. 2016; Peled et al. 2020).

Carbonic anhydrases (CAs), central to DIC speciation and carbon-concentrating mechanisms (CCM) that supply CO_2 for symbiont photosynthesis, commonly responding to environmental changes (Zoccola et al. 2015, 2016), were not among the DCPs in either species, consistent with other proteomic studies on corals (Lin et al. 2022). While CA gene expression and enzyme activities decreased in *S. pistillata* under long-term low pH (Zoccola et al. 2016), other studies showed varied responses from no impacts to increased expression (Hoadley et al. 2015; Vidal-Dupiol et al. 2013; Scucchia et al. 2021; Kenkel et al. 2018). These variations may reflect species-specific CCM regulation differences and reduced need for CA-mediated CO_2 supply under acidification and highlight the gap from gene expression level changes to proteomic and hence phenotypic outcomes. For calcification under OA, CAs may be less critical than assumed. Yet, other transporters may play important roles. Solute carrier (SLC) family proteins SLC8A1 and SLC3A1, increased in our study in *P. damicornis* and *S. pistillata*, respectively, are bicarbonate transporters associated with intracellular DIC delivery to photosymbionts, but some also deliver HCO_3^- to the site of calcification, and commonly respond to OA (Zoccola et al. 2015; Lin et al. 2022). Hence, they may fulfill an important function, alongside vesicle-mediated transport and matrix protein regulation (Leiva et al. 2023), and might take precedence in longer-term OA acclimatization.

These findings support that corals can modulate intracellular machinery and skeletal micro-environments to buffer against OA. However, such adjustments require energy and may only be sustainable in species with sufficient energetic reserves or photosynthetic support. Biological control of calcification is both a central mechanism of resilience and potential bottleneck under multi-stressor scenarios.

Molecular mechanisms and emerging themes of coral acclimatization to OA

Previous studies on both species found that DNA methylation increases with decreasing pH (Putnam et al. 2016; Liew et al. 2018), but fewer biomineralization-related genes were altered than expected, mainly affecting organic and cellular matrix components, which suggests a change in their scaffolding structures. Instead, mitogen-activated protein kinase (MAPK) signaling and cell growth pathways changed through variations in the JNK (c-Jun N-terminal kinase) pathway, delaying cell division and increasing cell and body growth (Liew et al. 2018). While a universal 'biomineralization toolkit' is unlikely (Gold and Vermeij 2023), common functional protein groups—such as vWF-domain adhesion proteins, protease inhibitors, and enzymes—appear critical

to coral calcification across species. These components may assist nucleation and OM organization, with OM production thought to lower aragonite crystal growth costs (Tambutté et al. 2015; Zaquin et al. 2022). In *S. pistillata*, we observed increased abundance of few stress-related proteins (glutathione hydrolase GSH, apoptotic markers) coupled with depletion of MAPK/JNK signaling proteins. These changes reflect reorganization of growth and skeletal morphology, consistent with polyp upscaling and increased skeletal porosity linked to epigenetic changes under OA (Liew et al. 2018). Such responses maintain linear extension despite reduced calcification efficiency. Consistent upregulation of Myosin-2 in acroporid populations from CO₂ seep environments (Kenkel et al. 2018; Leiva et al. 2023) and both studied species may reflect a conserved mechanism for OM delivery and cytoskeletal remodeling under stress. In aposymbiotic cold-water coral *Lophelia pertusa*, long-term low pH led to no differential CA expression, but colonies maintaining calcification showed unique overexpression of genes for proton transport and formation of microtubules and OM (Glazier et al. 2020), further supporting their relevance to OA resilience.

Our results align with understanding that coral acclimatization involves complex cellular strategies—spanning OM production, cytoskeletal restructuring, and ion regulation. These mechanisms at the proteomic level offer insight into reef-building corals' response to sustained ocean acidification.

Conclusions

Our study is among the first to integrate high-throughput proteomics with physiologic data on corals subjected to multiple long-term *p*CO₂ treatments. It provides insights into cellular mechanisms underlying coral responses to ocean acidification, highlighting species-specific proteomic signatures that diverge from canonical expectations. Contrary to assumptions that OA universally induces upregulation of proton pumps and ion transporters, our results show these responses are more nuanced and context-dependent. The differential proteomic responses between species underscore the importance of cellular plasticity in coral resilience. While *S. pistillata* adopts a dose-dependent metabolically intensive but flexible response strategy, *P. damicornis* maintains a more restrained molecular profile that might be advantageous under stable conditions but offers limited protection against compounding stressors. These differences between closely related pocilloporid coral species highlight the importance of biodiversity for reef resilience under future ocean scenarios.

This study demonstrates the value of proteomic approaches in coral ecophysiology. Unlike holobiont-level physiological

metrics alone, high-resolution compartment-specific proteomic data provide mechanistic clarity on pathways that corals deploy—or suppress—in response to environmental change. By linking protein-level adjustments with organismal outcomes, such as calcification and bleaching, proteomics enables a more nuanced understanding of coral resilience and susceptibility to climate change (Mayfield et al. 2022). This should yield additional avenues for reef restoration efforts and active interventions, such as human-assisted gene flow, selective breeding of corals, and ecosystem co-design (Westphal et al. 2022; Voolstra et al. 2023) applied toward coral reef management in a rapidly changing ocean environment.

Acknowledgements MS acknowledges the support by the Minerva Foundation (Max Planck Society) during phases of data analyses. This research was supported by baseline funding of ZMT to HW. LK and AS acknowledge the support by the "Ministerium für Kultur und Wissenschaft des Landes Nordrhein-Westfalen" and the "Bundesministerium für Forschung, Technologie und Raumfahrt". JR acknowledges support from the Hanse-Wissenschaftskolleg, the ZMT, and the US National Science Foundation (grant OCE-1437371) while conducting the *p*CO₂ experiments on the corals at the ZMT. We thank Louise Cameron (Northeastern University), the team of the Marine Experimental Facility at ZMT, specifically Nico Steinel and Silvia Hardenberg, for their enormous help during experiment preparations and long-term experimental aquarium maintenance. We are also grateful for the support of colleagues at ISAS, especially Ingo Feldmann.

Author contributions M.S., J.R., C.R. and H.W. contributed to conceptualization; M.S. and L.K. contributed to methodology; M.S. contributed to formal analysis, visualization, writing—original draft preparation and investigation; H.W. and A.S. contributed to resources; M.S. and L.K. contributed to data curation; H.W. supervised the study; J.R. and H.W. contributed to project administration; J.R. contributed to funding acquisition. M.S., D.dB., C.R., L.K., J.R., and H.W. contributed to writing—review and editing.

Funding Open Access funding enabled and organized by Projekt DEAL. Minerva Foundation, Ministerium für Kultur und Wissenschaft des Landes Nordrhein-Westfalen, Bundesministerium für Forschung, Technologie und Raumfahrt, Hanse-Wissenschaftskolleg, US National Science Foundation (Grant No. OCE-1437371)

Data availability All mass spectrometry data, including additional replicates and samples from the high *p*CO₂ treatment combined with thermal stress (31 °C), were deposited in the ProteomeXchange Consortium via the PRIDE partner repository (dataset identifier PXD062918). All data further analyzed and discussed here are also included in the electronic tables in the supplementary materials.

Declarations

Conflicts of interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Barott KL, Venn AA, Thies AB, Tambutté S, Tresguerres M (2020) Regulation of coral calcification by the acid-base sensing enzyme soluble adenyl cyclase. *Biochem Biophys Res Commun* 525:576–580
- Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. *Nature* 425:365
- Cameron LP, Reymond CE, Bijma J, Büscher JV, De Beer D, Guillermin M, Eagle RA, Gunnell J, Müller-Lundin F, Schmidt-Grieb GM, Westfield I, Westphal H, Ries JB (2022) Impacts of warming and acidification on coral calcification linked to photosymbiont loss and deregulation of calcifying fluid pH. *J Mar Sci Eng*. <https://doi.org/10.3390/jmse10081106>
- Capasso L, Ganot P, Planas-Bielsa V, Tambutté S, Zoccola D (2021) Intracellular pH regulation: characterization and functional investigation of H⁺ transporters in *Stylophora pistillata*. *BMC Mol Cell Biol*. <https://doi.org/10.1186/s12860-021-00353-x>
- Castillo KD, Ries JB, Bruno JF, Westfield IT (2014) The reef-building coral *Siderastrea siderea* exhibits parabolic responses to ocean acidification and warming. *Proc R Soc B: Biol Sci* 281:20141856
- Cohen AL, Holcomb M (2009) Why corals care about ocean acidification: uncovering the mechanism. *Oceanography* 22:118–127
- Comeau S, Cornwall CE, DeCarlo TM, Doo SS, Carpenter RC, McCulloch MT (2019) Resistance to ocean acidification in coral reef taxa is not gained by acclimatization. *Nat Clim Change* 9:477–483
- Cornwall CE, Comeau S, Kornder NA, Perry CT, van Hooidonk R, DeCarlo TM, Pratchett MS, Anderson KD, Browne N, Carpenter R, Diaz-Pulido G, D'Olivo JP, Doo SS, Figueiredo J, Fortunato SAV, Kennedy E, Lantz CA, McCulloch MT, González-Rivero M, Schoepf V, Smithers SG, Lowe RJ (2021) Global declines in coral reef calcium carbonate production under ocean acidification and warming. *Proc Natl Acad Sci USA*. <https://doi.org/10.1073/pnas.2015265118>
- Cornwall CE, Comeau S, Harvey BP (2024) Are physiological and ecosystem-level tipping points caused by ocean acidification? A critical evaluation. *Earth Syst Dyn* 15:671–687
- Costanza R, Arge R, Groot RD, Farber S, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Sutton P, van den Belt M, D'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Sutton P, van den Belt M (1997) The value of the world's ecosystem services and natural capital. *Nature* 387:253–260
- Cunning R, Bay RA, Gillette P, Baker AC, Traylor-Knowles N (2018) Comparative analysis of the *Pocillopora damicornis* genome highlights role of immune system in coral evolution. *Sci Rep* 8:16134
- Cziesielski MJ, Liew YJ, Cui G, Schmidt-Roach S, Campana S, Marondedze C, Aranda M (2018) Multi-omics analysis of thermal stress response in a zooxanthellate cnidarian reveals the importance of associating with thermotolerant symbionts. *Proc R Soc Lond B Biol Sci*. <https://doi.org/10.1098/rspb.2017.2654>
- Davies SW, Marchetti A, Ries JB, Castillo KD (2016) Thermal and pCO₂ stress elicit divergent transcriptomic responses in a resilient coral. *Front Mar Sci* 3:1–15
- Diz AP, Martínez-Fernández M, Rolán-Alvarez E (2012) Proteomics in evolutionary ecology: linking the genotype with the phenotype. *Mol Ecol* 21:1060–1080
- Gibbin EM, Davy SK (2014) The photo-physiological response of a model cnidarian-dinoflagellate symbiosis to CO₂-induced acidification at the cellular level. *J Exp Mar Biol Ecol* 457:1–7
- Glazier A, Herrera S, Weinnig A, Kurman M, Gómez CE, Cordes E (2020) Regulation of ion transport and energy metabolism enables certain coral genotypes to maintain calcification under experimental ocean acidification. *Mol Ecol* 29:1657–1673
- Gold DA, Vermeij GJ (2023) Deep resilience: an evolutionary perspective on calcification in an age of ocean acidification. *Front Physiol* 14:1–10
- Guillermin M, Cameron LP, De Corte I, Misra S, Bijma J, De Beer D, Reymond CE, Westphal H, Ries JB, Eagle RA (2021) Thermal stress reduces pocilloporid coral resilience to ocean acidification by impairing control over calcifying fluid chemistry. *Sci Adv*. <https://doi.org/10.1126/sciadv.aba9958>
- Helgoe J, Davy SK, Weis VM, Rodriguez-Lanetty M (2024) Triggers, cascades, and endpoints: connecting the dots of coral bleaching mechanisms. *Biol Rev* 99:715–752
- Herrera M, Liew YJ, Venn A, Tambutté E, Zoccola D, Tambutté S, Cui G, Aranda M (2021) New insights from transcriptomic data reveal differential effects of CO₂ acidification stress on photosynthesis of an endosymbiotic dinoflagellate *in hospite*. *Front Microbiol* 12:1–12
- Hoadley KD, Pettay DT, Grottoli AG, Cai WJ, Melman TF, Schoepf V, Hu X, Li Q, Xu H, Wang Y, Matsui Y, Baumann JH, Warner ME (2015) Physiological response to elevated temperature and pCO₂ varies across four Pacific coral species: understanding the unique host+symbiont response. *Sci Rep* 5:1–15
- Hoegh-Guldberg O, Poloczanska ES, Skirving W, Dove S (2017) Coral reef ecosystems under climate change and ocean acidification. *Front Mar Sci* 4:158
- Hohn S, Reymond CE (2019) Coral calcification, mucus, and the origin of skeletal organic molecules. *Coral Reefs* 38:973–984
- Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, Cook H, Mende DR, Letunic I, Rattei T, Jensen LJ, Von Mering C, Bork P (2019) EggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res* 47:D309–D314
- Hulver AM, Carbonne C, Teixidó N, Comeau S, Kemp DW, Keister EF, Gattuso JP, Grottoli AG (2024) Elevated heterotrophic capacity as a strategy for Mediterranean corals to cope with low pH at CO₂ vents. *PLoS ONE* 19:1–16
- IPCC I panel on climate change (2022) Sixth Assessment Report; Fact sheet Biodiversity, Climate change impacts risks. 1–3
- Jiang L, Sun YF, Zhou GW, Tong HY, Huang LT, Yu XL, Liu CY, Zhang YY, Yuan XC, Qian PY, Huang H (2022) Ocean acidification elicits differential bleaching and gene expression patterns in larval reef coral *Pocillopora damicornis* under heat stress. *Sci Total Environ* 842:156851
- Kaniewska P, Campbell PR, Kline DI, Rodriguez-Lanetty M, Miller DJ, Dove S, Hoegh-Guldberg O (2012) Major cellular and physiological impacts of ocean acidification on a reef building coral. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0034659>

- Kawahata H, Fujita K, Iguchi A, Inoue M, Iwasaki S, Kuroyanagi A, Maeda A, Manaka T, Moriya K, Takagi H, Toyofuku T, Yoshimura T (2019) Perspective on the response of marine calcifiers to global warming and ocean acidification — behavior of corals and foraminifera in a high CO₂ world “hot house.” *Prog Earth Planet Sci* 6:37
- Kenkel CD, Moya A, Strahl J, Humphrey C, Bay LK (2018) Functional genomic analysis of corals from natural CO₂-seeps reveals core molecular responses involved in acclimatization to ocean acidification. *Glob Chang Biol* 24:158–171
- Khalil M, Doo SS, Stühr M, Westphal H (2022) Ocean warming amplifies the effects of ocean acidification on skeletal mineralogy and microstructure in the asterinid starfish *Aquilonastra yairi*. *J Mar Sci Eng* 10:1065
- Leiva C, Pérez-Portela R, Lemer S (2023) Genomic signatures suggesting adaptation to ocean acidification in a coral holobiont from volcanic CO₂ seeps. *Commun Biol* 6:1–14
- Leung JYS, Zhang S, Connell SD (2022) Is ocean acidification really a threat to marine calcifiers? A systematic review and meta-analysis of 980+ studies spanning two decades. *Small*. <https://doi.org/10.1002/sml.202107407>
- Liew YJ, Zoccola D, Li Y, Tambutte E, Venn AA, Michell CT, Cui G, Deutekom ES, Kaandorp JA, Voolstra CR, Forêt S, Allemand D, Tambutte S, Aranda M (2018) Epigenome-associated phenotypic acclimatization to ocean acidification in a reef-building coral. *Sci Adv* 4:eaar8028
- Lin Z, Wang L, Chen M, Zheng X, Chen J (2022) Proteome and microbiota analyses characterizing dynamic coral-algae-microbe tripartite interactions under simulated rapid ocean acidification. *Sci Total Environ* 810:152266
- Maor-Landaw K, Levy O (2016) Survey of cnidarian gene expression profiles in response to environmental stressors: Summarizing 20 years of research. What are we heading for? In: Goffredo S, Dubinsky Z (eds) *The Cnidaria, Past, Present and Future: The world of Medusa and her sisters*. Springer International Publishing, Cham, pp 523–543
- Mass T, Putnam HM, Drake JL, Zelzion E, Gates RD, Bhattacharya D, Falkowski PG (2016) Temporal and spatial expression patterns of biomineralization proteins during early development in the stony coral *Pocillopora damicornis*. *Proc R Soc B* 283:20160322
- Mayfield AB (2022) Machine-learning-based proteomic predictive modeling with thermally-challenged Caribbean reef corals. *Diversity* 14:33
- Mayfield AB, Wang Y-B, Chen C-S, Chen S-H, Lin C-Y (2016) Dual-compartmental transcriptomic + proteomic analysis of a marine endosymbiosis exposed to environmental change. *Mol Ecol* 25:5944–5958
- Mollica NR, Guo W, Cohen AL, Huang KF, Foster GL, Donald HK, Solow AR (2018) Ocean acidification affects coral growth by reducing skeletal density. *Proc Natl Acad Sci U S A* 115:1754–1759
- Moya A, Huisman L, Ball EE, Hayward DC, Grasso LC, Chua CM, Woo HN, Gattuso JP, Forêt S, Miller DJ (2012) Whole transcriptome analysis of the coral *Acropora millepora* reveals complex responses to CO₂-driven acidification during the initiation of calcification. *Mol Ecol* 21:2440–2454
- Moya A, Huisman L, Forêt S, Gattuso JP, Hayward DC, Ball EE, Miller DJ (2015) Rapid acclimation of juvenile corals to CO₂-mediated acidification by upregulation of heat shock protein and Bcl-2 genes. *Mol Ecol* 24:438–452
- Narayan GR, Reymond CE, Stühr M, Doo S, Schmidt C, Mann T, Westphal H (2022) Response of large benthic foraminifera to climate and local changes: Implications for future carbonate production. *Sedimentology* 69:121–161
- Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnani-desikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner G-K, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig M-F, Yamanaka Y, Yool A (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437:681
- Peled Y, Drake J, Malik A, Almuly R, Lizarz M, Morgenstern D, Mass T (2020) Optimization of skeletal protein preparation for LC-MS/MS sequencing yields additional coral skeletal proteins in *Stylophora pistillata*. *BMC Mater* 2:15
- Putnam HM, Davidson JM, Gates RD (2016) Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. *Evol Appl* 9:1165–1178
- R Core Team (2016) R: A language and environment for statistical computing.
- Ries JB (2011) A physicochemical framework for interpreting the biological calcification response to CO₂-induced ocean acidification. *Geochim Cosmochim Acta* 75:4053–4064
- Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO₂-induced ocean acidification. *Geology* 37:1131–1134
- Rocker MM, Noonan S, Humphrey C, Moya A, Willis BL, Bay LK (2015) Expression of calcification and metabolism-related genes in response to elevated pCO₂ and temperature in the reef-building coral *Acropora millepora*. *Mar Genomics* 24:313–318
- Scucchia F, Malik A, Putnam HM, Mass T (2021) Genetic and physiological traits conferring tolerance to ocean acidification in mesophotic corals. *Glob Chang Biol* 27:5276–5294
- Stühr M, Blank-Landeshammer B, Reymond CE, Kollipara L, Sickmann A, Kucera M, Westphal H (2018) Disentangling thermal stress responses in a reef-calcifier and its photosymbionts by shotgun proteomics. *Sci Rep* 8:3524
- Stühr M, Cameron LP, Blank-Landeshammer B, Reymond CE, Doo SS, Westphal H, Sickmann A, Ries JB (2021) Divergent proteomic responses offer insights into resistant physiological responses of reef-foraminifera to climate change. *Oceans* 10:3390
- Stühr M, Blank-Landeshammer B, Meyer A, Baumeister V, Rahnenführer J, Sickmann A, Westphal H (2022) Proteome-based clustering approaches reveal phylogenetic insights into *Amphistegina*. *J Earth Sci* 33:1469–1479
- Takeuchi T, Yamada L, Shinzato C, Sawada H, Satoh N (2016) Stepwise evolution of coral biomineralization revealed with genome-wide proteomics and transcriptomics. *PLoS ONE* 11(11(6)):e0156424
- Tambutté E, Venn AA, Holcomb M, Segonds N, Techer N, Zoccola D, Allemand D, Tambutté S (2015) Morphological plasticity of the coral skeleton under CO₂-driven seawater acidification. *Nat Commun* 6:7368
- Towle EK, Enochs IC, Langdon C (2015) Threatened Caribbean coral is able to mitigate the adverse effects of ocean acidification on calcification by increasing feeding rate. *PLoS ONE* 10:1–17
- Tremblay P, Fine M, Maguer JF, Grover R, Ferrier-Pagès C (2013) Photosynthate translocation increases in response to low seawater pH in a coral-dinoflagellate symbiosis. *Biogeosciences* 10:3997–4007
- Venn AA, Tambutté E, Caminiti-Segonds N, Techer N, Allemand D, Tambutté S (2019) Effects of light and darkness on pH regulation in three coral species exposed to seawater acidification. *Sci Rep* 9:1–12
- Vidal-Dupiol J, Zoccola D, Tambutté E, Grunau C, Cosseau C, Smith KM, Freitag M, Dheilly NM, Allemand D, Tambutté S (2013) Genes related to ion-transport and energy production are upregulated in response to CO₂-driven pH decrease in corals: new insights from transcriptome analysis. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0058652>
- Vizcaíno JA, Csordas A, Del-Toro N, Dienes JA, Griss J, Lavidas I, Mayer G, Perez-Riverol Y, Reisinger F, Ternent T, Xu QW, Wang

- R, Hermjakob H (2016) 2016 update of the PRIDE database and its related tools. *Nucleic Acids Res* 44:D447–D456
- Voolstra CR, Li Y, Liew YJ, Baumgarten S, Zoccola D, Flot JF, Tambutté S, Allemand D, Aranda M (2017) Comparative analysis of the genomes of *Stylophora pistillata* and *Acropora digitifera* provides evidence for extensive differences between species of corals. *Sci Rep* 7:1–14
- Voolstra CR, Peixoto RS, Ferrier-Pagès C (2023) Mitigating the ecological collapse of coral reef ecosystems. *EMBO Rep* 24:1–7
- Wahl M, Saderne V, Sawall Y (2016) How good are we at assessing the impact of ocean acidification in coastal systems? Limitations, omissions and strengths of commonly used experimental approaches with special emphasis on the neglected role of fluctuations. *Mar Freshw Res* 67:25–36
- Westphal H, Murphy GN, Doo SS, Mann T, Petrovic A, Schmidt C, Stühr M (2022) Ecosystem design as an avenue for improving services provided by carbonate producing marine ecosystems. *PeerJ* 10:1–30
- Williams A, Stephens TG, Shumaker A, Bhattacharya D (2023) Peeling back the layers of coral holobiont multi-omics data. *iScience* 26:107623
- Zaquin T, Di Bisceglie AP, Pinkas I, Falini G, Mass T (2022) Different skeletal protein toolkits achieve similar structure and performance in the tropical coral *Stylophora pistillata* and the temperate *Oculina patagonica*. *Sci Rep* 12:1–11
- Zoccola D, Ganot P, Bertucci A, Caminiti-Segonds N, Techer N, Voolstra CR, Aranda M, Tambutté E, Allemand D, Casey JR, Tambutté S (2015) Bicarbonate transporters in corals point towards a key step in the evolution of cnidarian calcification. *Sci Rep* 5:1–11
- Zoccola D, Innocenti A, Bertucci A, Tambutté E, Supuran CT, Tambutté S (2016) Coral carbonic anhydrases: regulation by ocean acidification. *Mar Drugs* 14:109
- Zybailov B, Mosley AL, Sardu ME, Coleman MK, Florens L, Washburn MP (2006) Statistical analysis of membrane proteome expression changes in *Saccharomyces cerevisiae*. *J Proteome Res* 5:2339–2347

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.